# A HEREDITARY VARIEGATION IN TOMATOES[1]

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Received April 17, 1942

FOR several years an occasional variegated tomato plant has been observed in our field cultures. The variegation often appears to be due to bud variation, since one part of the plant is variegated while the rest seems to be normal.

The upper surface of a variegated leaflet is composed of normal green and abnormal pale-green portions (fig. 1, C). The pale-green color of the abnormal portions resembles that of the lower surface of a normal leaf, which is decidedly paler than the upper surface. Pale-green leaflets are smoother than normal leaflets and are often rolled upwards. The area of the pale portion is reduced, so that the halves of a leaflet on either side of the midrib may be unequal, or the lamina may be distorted (fig. 1, A).

The distribution of normal and pale-green areas is very irregular. On a variegated shoot, the pale condition may affect a whole leaf, all the leaflets on one side of the rachis, one half of a leaflet at one side of the midrib, or a very small patch of the lamina. The stem is usually marked with alternating normal green and pale-green stripes, which vary in length and in width (fig. 1, B). A stripe may be broad, almost completely enveloping the stem, or it may be very narrow, constituting a mere striation. A stripe may be confined to a single internode, or it may be longer, passing upward into an inflorescence or into another internode. Leaves and stems which at first glance seem to be wholly pale, usually have small areas of normal tissue.

In the field, the leaves of much-variegated shoots look somewhat like those of the gene mutant wilty (MACARTHUR 1934). The leaves of wilty (*wt wt*) and of variegated plants are much less uprolled in the greenhouse; consequently, the spotty appearance of some variegated leaves is more conspicuous there than in the field. The upper surface of the leaves of wilty is normal green.

Purple anthocyanin develops in the stem tips and petioles of plants containing the normal allele *A*1 (MACARTHUR 1931), especially in cool weather and with exposure to direct sunlight. On variegated shoots, when anthocyanin is abundant, there is a deficiency of purple in the pale portions, and purple and nonpurple stripes occur side by side on the young stem. A little brownish-purple pigment, usually limited to thin lines, may occur in the petioles of wholly pale leaflets. The variegation therefore affects both the green (chlorophyll) and the purple (anthocyanin) pig-

[1] Paper No. 457, UNIVERSITY OF CALIFORNIA CITRUS EXPERIMENT STATION, Riverside, California.

mentation of the plant. The difference in color and in smoothness of leaf surface caused by variegation, is best seen in dwarf ($d_1d_1$) plants which contain the gene $A_1$ for purple anthocyanin and have made nearly their

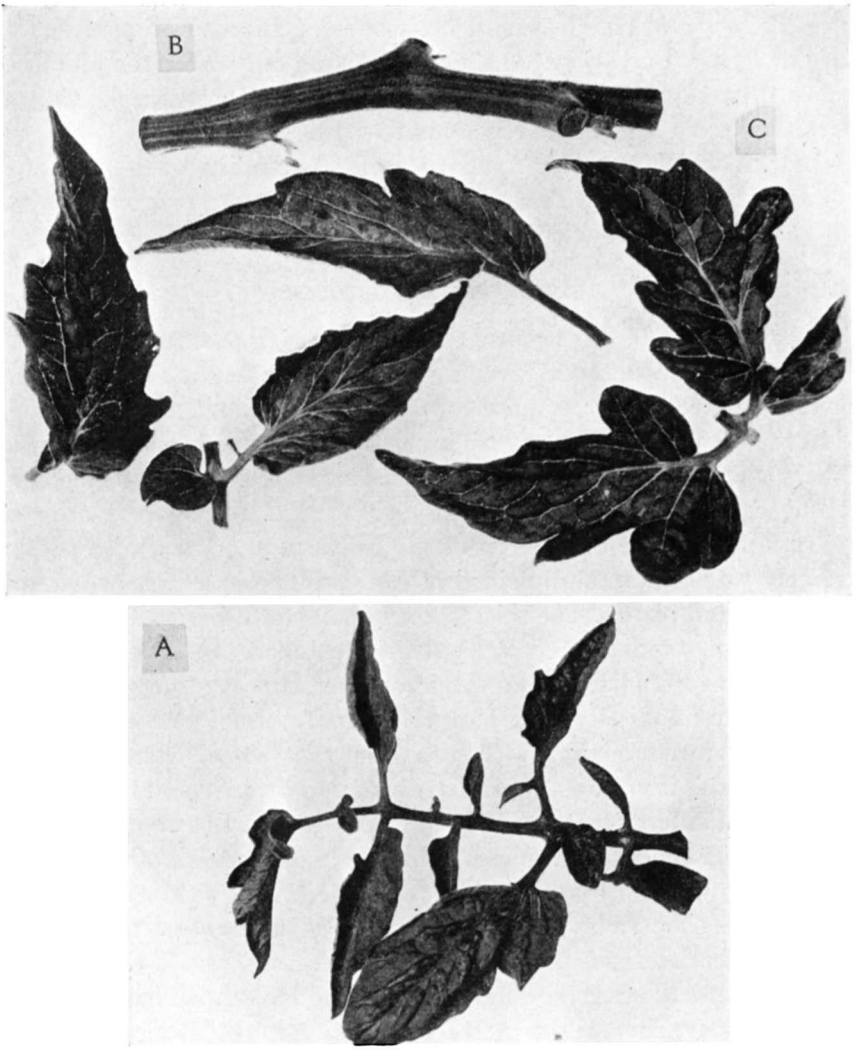



FIGURE 1.—Stem and leaves of a variegated tomato plant. Abnormal tissue is paler than normal tissue. A, leaf: large leaflet below is normal; smaller leaflets are uprolled and mostly composed of abnormal tissue. B, variegated stem. C, leaflets: upper one of pair at right has abnormal patches; two in the center are largely abnormal; leaflet at left is mostly normal.

full vegetative growth and have not been pruned. No variegation has been observed in unripe or ripe fruits.

In one population derived from selfing a much-variegated plant, practically every shoot became much variegated. One plant was much stunted

and distorted, as if affected with cucumber mosaic, and was entirely unfruitful. The others were very unproductive. One of these much-variegated plants was crossed with a nonvariegated male. Two weeks after the seeds resulting from this cross had been planted, variegation was perceptible in the cotyledons and true leaves of the seedlings. In populations from less-variegated parents, variegated shoots appeared only after the plants had reached their maximum size, two or three months after transplanting; the rest of the leaves and shoots remained normal.

A branch which has abnormal pale areas extending over most of the stem and leaves, is, as a rule, unfruitful. On the same plant a normal or slightly variegated branch is, as a rule, fruitful.

## CYTOLOGY AND HISTOLOGY

Normal tomato flowers occur on branches having normal leaves and are a bright clear yellow when mature. Flowers on a branch which has abnormal pale-green stems and leaves, remain pale yellow, never open fully, and have pale thin anthers containing little or no pollen.

The pollen from variegated plants is characterized by irregularity in size and by the presence of abnormally large grains, which appear to result from complete failure of division of the pollen mother cell. Such pollen is extremely rare in normal diploids but can be found in nearly every smear from variegated plants. The microspores of the tetrad, instead of forming a tetrahedron, tend to be arranged in one plane. Tetrads from badly affected plants often have unequal microspores, and dyads or pentads seem to occur more frequently than in anthers from normal plants. Except for occasional non-reduction, which also occurs to some extent in normal diploids, meiosis is normal. There are 12 pairs of chromosomes as in normal diploids. The difference in cytological behavior seems to be due to an abnormal condition in the cytoplasm, which prevents cytokinesis or makes it slow or irregular.

In a normal shoot, a layer of palisade cells lies just beneath the epidermis (fig. 2, A). In the developing shoot of a variegated plant, some of these cells fail to undergo normal differentiation and look much like the cells of the spongy parenchyma (fig. 2, B). For this reason, the upper surface of the abnormal portion of a variegated leaf is gray-green and resembles the lower surface in color. Low magnification of the upper surface of the normal portion of a variegated leaf shows a large number of small cell ends so closely massed as to cause buckling and to partially obscure the veins. In the abnormal portion of the leaf, large, irregular, loosely arranged cells can be seen beneath the epidermis. Sections of variegated leaves show that the gray-green areas lack the palisade layer (fig. 2, B). The irregularly shaped cells contain plastids, but since relatively few cells are present in

abnormal regions, much less chlorophyll is present than in normal portions of the leaf. In abnormal regions, starch grains have been observed in the chloroplasts, and the latter appear to be normal in color. In the pale-green area of a variegated leaf, the outer periblem layer of the upper surface is either modified to form spongy parenchyma or is absent.

In normal regions of the stems, two to four layers of chlorenchyma lie immediately below the epidermis. Some of these cells may contain purple

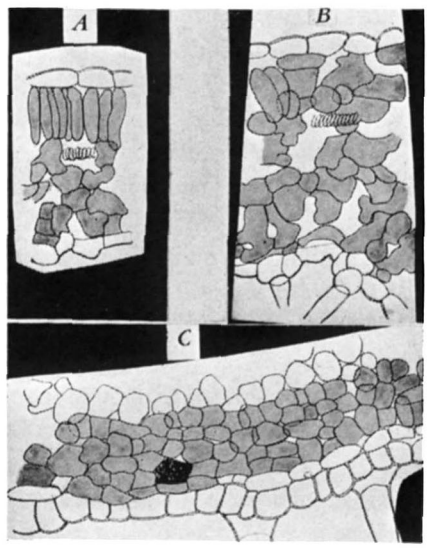



FIGURE 2.—Drawings from freehand sections of fresh tomato leaves and stem. A, normal-green portion of a leaf. B, pale-green portion of a leaf. C, portion of variegated stem including one normal-green and two pale-green stripes; the subepidermal cells in the pale regions lack chlorophyll. ×360.

anthocyanin, especially on the side of the stem exposed to direct sunlight. In the abnormal regions, one or, occasionally, two layers of large flat cells lie just beneath the epidermis. These cells contain no chloroplasts, and neither they nor the two or three layers of chlorenchyma beneath them develop anthocyanin (fig. 2, C). Except for the presence of this colorless subepidermal layer, which gives a gray-green cast to the abnormal stem stripes, the stems are normal. Occasionally, the chlorenchyma from a normal region of the stem tends to slightly overgrow the abnormal subepidermal layer.

## TRANSMISSION TESTS

It was at first suspected that the variegation was due to a virus, but its appearance did not correspond to that of any known virus disease. DR. S. P. DOOLITTLE, Senior Pathologist of the UNITED STATES DEPARTMENT

OF AGRICULTURE, made infection tests at Washington, D. C., by rubbing the leaves of healthy tobacco and tomato plants with juice from tissue of variegated tomato plants and by inserting fragments of this tissue into the stems. Ordinary tobacco mosaic was recovered from one variegated plant, but in no case were the peculiar symptoms of variegation transmitted to normal tomato plants. (Leaves of tomato plants affected with tobacco mosaic develop a palisade layer and so differ from those of the variegated tomato.)

Subsequently, at Riverside, variegated scions were successfully grafted onto three normal plants, and normal scions were successfully grafted onto two variegated plants. No symptoms of variegation were found on the shoots of the normal plants and normal scions, even after stock and scion had been growing together for three months.

The nontransmission of the variegation by these means indicates strongly that it is not caused by an ordinary virus. Whole families consisting of many hundreds of plants growing in rows adjacent to variegated plants have remained free from variegation.

## GENETICS OF VARIEGATION

Most of the variegated plants can be traced to a simple trisomic plant, triple-H (triplo-IV), C218-8-1, raised in 1932, which has been crossed with various unrelated plants, but a few authentic cases appear to have an independent origin. Variegation was not systematically recorded until September, 1939. The number of variegated plants at that time was probably underestimated, since in some cases variegation was very slight and easily overlooked. In 1940, and especially in 1941, a more careful search was made, so that the records for those years are more complete. For brevity, "V" and "NV" will be used to indicate "variegated" and "nonvariegated," respectively.

Breeding data of V and NV plants are presented in table 1, and the pedigrees of the plants concerned are shown in figures 3, 4, and 5.

A majority of the plants in three small families from selfing parents C373-1, C373-2, and C373-3 (see table 1 and fig. 4), grown in 1939, were variegated. Two families from V $F_2$ parents C373-2-1 and C373-2-2, contained 25 plants, all of which were variegated. Apparently, V and NV plants segregated out in two other $F_3$ families from parents C324-2-1 and C324-2-2, V plants again predominating. Two $F_1$ families, C430 (fig. 3), from V ♀ pollinated by NV? (probably nonvariegated) ♂, and C519 (fig. 3), from V×NV, consisted wholly of V plants. These data suggest that variegation depends on a gene or on genes and is dominant.

In 1940 and 1941, however, two $F_2$ families from the sibs C430-1 and C430-2, consisting of 43 and 24 plants, respectively, were all variegated.

TABLE 1

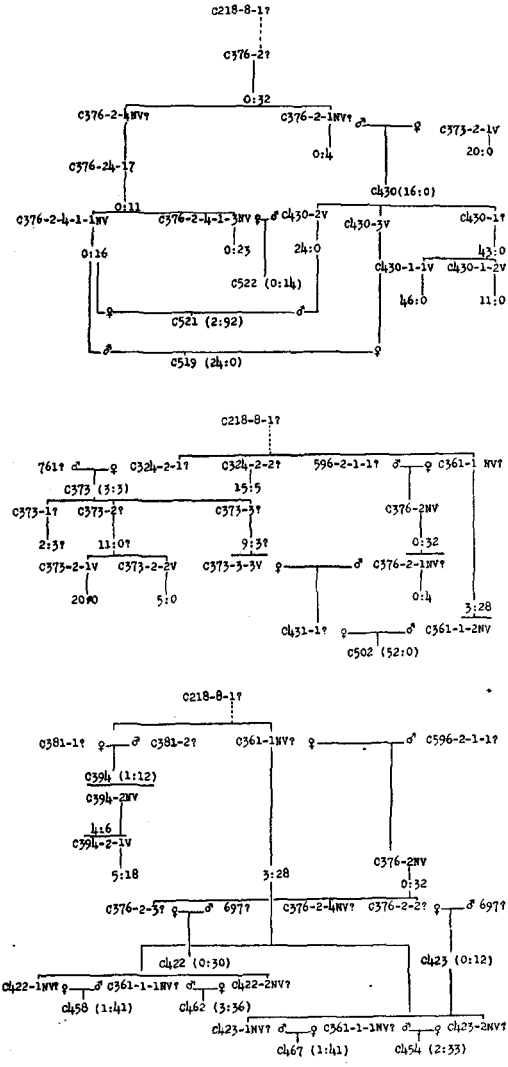
*Inheritance of variegation in tomatoes. V = variegated; NV = nonvariegated; ? = doubt as to the phenotype.*

| PARENT | | PROGENY | | | |
|---|---|---|---|---|---|
| PEDIGREE | PHENOTYPE | YEAR RECORDED | GENERATION | V | NV |
| C373-1 | ? | 1939 | $F_2$ | 2 | 3? |
| C373-2 | ? | 1939 | $F_2$ | 11 | 0? |
| C373-3 | ? | 1939 | $F_2$ | 9 | 3? |
| C373-2-1 | V | 1940 | $F_3$ | 20 | 0 |
| C373-2-2 | V | 1940 | $F_3$ | 5 | 0 |
| C324-2-1 | ? | 1939 | $F_3$ | 25 | 10 |
| C324-2-2 | ? | 1940 | $F_3$ | 15 | 10 |
| C430 | V×NV? | 1940 | $F_1$ | 16 | 0 |
| C519 | V×NV | 1941 | $F_1$ | 24 | 0 |
| C536 | V×NV | 1942 | $F_1$ | 32 | 0 |
| C430-1 | ? | 1940 | $F_2$ | 43 | 0 |
| C430-2 | V | 1941 | $F_2$ | 24 | 0 |
| C430-1-1 | V | 1941 | $F_3$ | 46 | 0 |
| C430-1-2 | V | 1941 | $F_3$ | 11 | 0 |
| C502 | ?×NV | 1941 | Backcross | 52 | 0 |
| C521 | NV×V | 1941 | $F_1$ | 2 | 92 |
| C522 | NV×V | 1941 | $F_1$ | 0 | 14 |
| C394 | ?×? | 1939 | $F_1$ | 1 | 12 |
| C394-2 | NV | 1940 | $F_2$ | 4 | 6 |
| C394-2-1 | V | 1941 | $F_3$ | 5 | 18 |
| C361-1 | NV? | 1940 | $F_2$ | 3 | 28 |
| C454 | NV?×NV? | 1940 | $F_1$ | 2 | 33 |
| C458 | NV?×NV? | 1940 | $F_1$ | 1? | 41 |
| C462 | NV?×NV? | 1940 | $F_1$ | 3 | 36 |
| C467 | NV?×NV? | 1940 | $F_1$ | 1 | 41 |
| C376-2 | NV | 1939 | $F_2$ | 0 | 32 |
| C376-2-1 | NV? | 1940 | $F_3$ | 0 | 4 |
| C376-2-4-1 | NV? | 1940 | $F_4$ | 0 | 11 |
| C376-2-4-1-1 | NV | 1941 | $F_5$ | 0 | 16 |
| C376-2-4-1-3 | NV | 1941 | $F_5$ | 0 | 23 |

One of the $F_1$ parents, C430-2, and all its $F_2$ progeny were extremely variegated and highly unfruitful. The phenotype of C430-1 is uncertain, as this plant was grown under glass and drastically pruned. Two V $F_2$ plants, C430-1-1 and C430-1-2, gave, in $F_3$, only V progeny. A V×NV $F_1$ backcrossed with NV gave 52 V plants (C502, fig. 4). No NV plants occurred in any of these $F_2$, $F_3$, or backcross families.

In 1941, two $F_1$ families, C521 and C522 (fig. 3), which were from NV ♀ parents of the same family, crossed with the same very much variegated plant, C430-2, contained 106 NV and only two slightly V plants. The two NV ♀ parents, C376-2-4-1-1 and C376-2-4-1-3, belonged to a family of

11 NV plants; and C376-2-4, the grandparent of this family, grown in 1939, was probably nonvariegated (fig. 3). V plants did occur, however, in the progeny from selfing a $P_1$ grandparent, C361-1, of this $F_2$ (fig. 5), and




FIGURES 3–5. (Figure 3 top, figure 5 bottom).—Pedigrees of tomato plants. The ratio of variegated to nonvariegated plants is shown under the series number of the parent plant or in parentheses after the series number of an $F_1$ family. "V" indicates a variegated parent; "NV" indicates a nonvariegated parent; "?" indicates some doubt as to parent phenotype.

it is possible that variegation was introduced by the ♀ parents of crosses C521 and C522.

These later, more complete data do not indicate that variegation depends on a dominant gene or genes, since there is no evidence of segregation

of genes for variegation in the $F_2$, $F_3$, and backcross families. Apparently, all or nearly all the megaspores of a variegated plant, but very few of the pollen grains, transmitted variegation. The most probable hypothesis seems to be that variegation is inherited through the cytoplasm and is, as a rule, maternally inherited.

Several families other than C521, which has already been mentioned, contained a minority of V plants. The phenotypes of most of the parents are not known. Many were grown in 1939, when slight variegation is likely to have been overlooked. In 1939, an $F_1$ family, C394, from unknown parent phenotypes, contained one V and 12 NV plants (fig. 5); and an $F_2$ from an NV $F_1$ parent, C394-2, contained a slight excess of NV plants. An $F_3$ from a V $F_2$ plant, C394-2-1, contained five V and 18 NV plants. Plant C361-1 (fig. 5), which was heterozygous for a deficiency and very slow-growing (Lesley and Lesley 1941), was probably nonvariegated. When this plant was selfed, only three of its 31 progeny were variegated. Four $F_1$ families from crosses C454, C458, C462, and C467 (fig. 5), whose parents are supposed to have been nonvariegated, also contained a minority of V plants. They had one parent in common, C361-1-1, which had V sibs; the other parents were also related to the deficient plant, which, as noted above, had a few V progeny. All the families containing a minority of variegated plants therefore originated from parents which were either variegated or related to variegated plants. Very slight variegation affecting only a few cells may easily have been overlooked in the supposed NV plants. On the whole, the evidence suggests that slightly V or even NV parents, when selfed or crossed with other NV plants, may give both NV and V progeny.

## DISCUSSION

The palisade layer, which lies just beneath the upper epidermis of a normal leaf, is largely replaced in the abnormal portions of variegated leaves by irregular cells which resemble the spongy parenchyma.

A similar lack of palisade layer was observed by FUNAOKA (1924) in the pale portions of variegated *Acer Negundo* and *Glechoma hederaceum*. CONTI (1934), in a study of 37 plant species having typical hereditary white mottling, found that where chloroplasts were absent, there was a marked modification of the palisade layer in 18 species and a classical palisade structure in the other 19.

In the variegated tomato, the presence of small islands or isolated strips of normal cells in otherwise abnormal tissue, or *vice versa*, suggests that the change may proceed in either direction.

According to KÜSTER (1903, 1927), palisade tissue may be lacking as a result of shading, growth in artificial illumination, growth in extreme humidity or with leaves submerged, or as a result of fungal infection.

EAMES and MACDANIELS (1925) state that in normal plants the number of palisade layers and the density of the cell structure depend largely, either directly or indirectly, on light intensity. There may thus be a great variation in the proportion and arrangement of the palisade parenchyma in the same species growing under different conditions. There may also be considerable variation in mesophyll structure of leaves from different parts of the same plant. Since one part of a variegated tomato leaf may lack palisade cells while an adjacent region is normal, and since the abnormal condition is multiplied and transmitted somatically and is inherited, the condition which produces it must be within the cell and cannot be attributed to these external factors. The abnormal cytokinesis of the pollen mother cells on variegated plants seems to indicate that the cytoplasm is in an abnormal condition or that normal control over the cytoplasm is lacking.

According to LANGE (1927), in Solanum the germ cells originate from the subepidermal layer. The abnormality of this cell layer in the variegated tomato is certainly hereditary. That inheritance is predominantly maternal is indicated by the fact that no normal plants occurred in several carefully examined $F_2$ and backcross families from variegated ♀ parents. The variable proportions of variegated and nonvariegated plants found in some families and the instability of the variegation itself as it occurs on the plant also favor this hypothesis. The fact that variegation occurs earlier and develops more extensively in the progeny of a much-variegated seed parent than in that of a slightly variegated parent, is suggestive of a variegation inherent in the cytoplasm. Nevertheless, a gene interpretation is not excluded. Nontransmission by the pollen might be due to a pollen-lethal gene, and the scarcity of nonvariegated plants in the $F_2$ and backcross families might be due to the existence of several dominant genes for variegation. It is possible, also, that the normal $F_1$ plants from crosses $C_{521}$ and $C_{522}$ (fig. 3) would give variegated plants in $F_2$.

Since the flowers of predominantly pale shoots are sterile, variegated plants are usually reproduced by seeds from mixed or normal-looking shoots. Variegated plants apparently develop from all the egg cells, even of a ♀ parent of which some shoots appear to be nonvariegated. Because of somatic segregation, it may be possible to increase or decrease the amount of variegation by bud selection. At present the most promising method of eliminating variegation from a variety is by crossing it with a nonvariegated race and by repeated backcrossing, the variegated variety being used as pollen parent.

The plastids are the only known agents in cytoplasmic inheritance. Some relation between variegation and plastid distribution was accordingly suspected. In the subepidermal cells of the pale areas of the leaf, there are probably fewer chloroplasts than in the palisade cells of a normal area. In

the pale portions of the stem, the cells of the subepidermal layer are modified in shape and lack both chloroplasts and anthocyanin.

CHITTENDEN (1927) adduces evidence that, in some cases of mosaic anthocyanin distribution, the presence of anthocyanin depends on the activity of the plastids, and that in such plants maternal inheritance through plastids is more probable than inheritance by genes. In the variegated tomato inheritance of mosaic anthocyanin distribution appears to be cytoplasmic, and the pale-green color and lack of purple anthocyanin seem to depend on a deficiency of chloroplasts. Possibly the lack of anthocyanin is caused by insufficient glucose, two molecules of which enter into the formation of anthocyanin.

It appears that the presence of purple anthocyanin is determined by the independent genes *A*1 and *a*2 (MACARTHUR 1934) and by the chloroplasts. In the tomato chimera with variegated fruit, described by MACARTHUR (1928), which seems to be a typical case of plastid inheritance, purple anthocyanin color is not affected. However, the modified cell form of the subepidermal layer in abnormal regions of both stem and leaf, the abnormal cytokinesis in the pollen mother cells, and the fact that green plastids are present in the subepidermal cells of the leaf, suggest that in the present instance, variegation is due to an unstable or a "diseased" condition of the cytoplasm, as postulated by CORRENS (1937), which affects the formation of chlorophyll and anthocyanin. It seems probable that a certain specific portion of the egg cytoplasm may form the subepidermal layer and is abnormal in contrast to the rest of the cytoplasm.

## SUMMARY

A variegation of tomatoes is described, in which abnormal pale-green areas occur on normal green stems and leaves. The pattern of pale-green and normal tissues on a variegated plant is very irregular, and the pale-green area is extremely variable in extent. On a predominantly pale shoot, flower development is arrested, cytokinesis is irregular, and the pollen is very scanty. In extreme cases the entire plant is unfruitful. Variegated plants which have the purple anthocyanin gene *A*1 have no anthocyanin in the pale portions of the stem and very little in the petioles of pale leaves.

In the pale portion of the leaf, palisade cells are absent, so that the upper surface is similar in color to the lower leaf surface. In the stem, the cells of the subepidermal layer of the chlorenchyma are abnormally flattened and contain no chloroplasts.

The variegation was not transmissible by the methods ordinarily effective with a virus and did not spread to adjacent unrelated families.

Variegation behaves as a dominant character. It is, as a rule, inherited maternally and appears to have its basis in the cytoplasm. Some parents

which were not known to be variegated gave a small proportion of variegated progeny. The occurrence of about two percent of variegated plants from nonvariegated ♀ × variegated ♂ may therefore be due to variegation introduced by female parents in which variegation was overlooked.

The absence of chloroplasts in the subepidermal layer of the stems of variegated plants may account for the lack of anthocyanin in the abnormal areas. The histological and cytological changes found in the pale portions of variegated plants, and the mode of inheritance of variegation, indicate that variegation is due to an abnormality of the cytoplasm.

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